L Number	Hits	Search Text	DB	Time stamp
1	2	("5770414").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 16:53
7	14	human NEAR neur$10 ADJ progenitor	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 17:01
14	5	human NEAR mesencephalon	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 17:03
20	23	(US-5580777-$ or US-5750376-$ or US-5753506-$ or US-5766948-$ or US-5770414-$ or US-5851832-$ or US-5980885-$ or US-5981165-$ or US-6013521-$ or US-6040180-$ or US-6020197-$ or US-6045807-$ or US-6071889-$ or US-6197585-$ or US-6265175-$ or US-6294346-$ or US-5411883-$ or US-6284539-$).did. or (US-20020009743-$ or US-20020039789-$ or US-20020006660-$).did. or (WO-9949014-$ or WO-200009669-$).did.	USPAT; US-PGPUB; DERWENT	2002/09/17 17:04
-	287	mesencephalon	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:09
-	268	mesencephalon and (neuron$10 or neural$5)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:34
-	171	(mesencephalon and (neuron$10 or neural$5)) and (precursor or progenitor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:11
-	51	(((mesencephalon and (neuron$10 or neural$5)) and (precursor or progenitor)) and (FGF$3 EGF PDGF CNTF IFG$5 BDNF)) and (immortal$10)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:13
-	252	(mesencephalon and (neuron$10 or neural$5)) and human	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:34
-	5	(mesencephalon and (neuron$10 or neural$5)) and (human NEAR mesencephalon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:36
-	2	mesencephalon and SAH	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:36
-	110	((mesencephalon and (neuron$10 or neural$5)) and (precursor or progenitor)) and (FGF$3 EGF PDGF CNTF IFG$5 BDNF)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:41
-	16	((mesencephalon and (neuron$10 or neural$5)) and human) and mesencephalon.clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:42

-	1	((US-5580777-$ or US-5750376-$ or US-5753506-$ or US-5766948-$ or US-5770414-$ or US-5851832-$ or US-5980885-$ or US-5981165-$ or US-6013521-$ or US-6040180-$ or US-6020197-$ or US-6045807-$ or US-6071889-$ or US-6197585-$ or US-6265175-$ or US-6294346-$ or US-5411883-$ or US-6284539-$).did. or (US-20020009743-$ or US-20020039789-$).did.) and (human NEAR mesencephalon)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:45
-	9	((US-5580777-$ or US-5750376-$ or US-5753506-$ or US-5766948-$ or US-5770414-$ or US-5851832-$ or US-5980885-$ or US-5981165-$ or US-6013521-$ or US-6040180-$ or US-6020197-$ or US-6045807-$ or US-6071889-$ or US-6197585-$ or US-6265175-$ or US-6294346-$ or US-5411883-$ or US-6284539-$).did. or (US-20020009743-$ or US-20020039789-$).did.) and mesencephalon.clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:46
-	12	((US-5580777-$ or US-5750376-$ or US-5753506-$ or US-5766948-$ or US-5770414-$ or US-5851832-$ or US-5980885-$ or US-5981165-$ or US-6013521-$ or US-6040180-$ or US-6020197-$ or US-6045807-$ or US-6071889-$ or US-6197585-$ or US-6265175-$ or US-6294346-$ or US-5411883-$ or US-6284539-$).did. or (US-20020009743-$ or US-20020039789-$).did.) and v-myc	USPAT; US-PGPUB; EPO; JPO; DERWENT	2002/09/17 14:47

CORRECTED VERSION*

PCT

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International Bureau

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A1

(11) International Publication Number: WO 00/09669

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(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
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(54) Title: HUMAN MESENCEPHALON CELL LINES AND METHODS OF USE THEREFOR

(57) Abstract

Conditionally-immortalized human mesencephalon cell lines are provided. Such cell lines, which may be clonal, may be used to generate neurons, including dopaminergic neurons. The cell lines and/or differentiated cells may be used for the development of therapeutic agents to prevent and treat a variety of neurological diseases such as Parkinson's disease. The cell lines and/or differentiated cells may also be used in assays and for the general study of mesencephalon cell development and differentiation.

*(Referred to in PCT Gazette No. 31/2000, Section II)

(FILE 'HOME' ENTERED AT 16:14:55 ON 17 SEP 2002)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, EMBASE, BIOSIS, MEDICONF' ENTERED AT 16:15:03 ON 17 SEP 2002

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L2          10 DUP REM L1 (13 DUPLICATES REMOVED)
L3          10 SORT L2 PY
L4       31741 S MESENCEPHALON
L5         208 S L4 AND (NEUR? (S) PROGENITOR)
L6          87 DUP REM L5 (121 DUPLICATES REMOVED)
L7           5 S L6 AND IMMORTAL?
L8           5 SORT L7 PY
L9       11273 S NEUR? (S) PROGENITOR
L10        440 S L9 AND IMMORTAL?
L11        141 DUP REM L10 (299 DUPLICATES REMOVED)
L12        141 FOCUS L11 1-
L13          5 S L11 AND HC2S2
L14       1417 S L9 AND (FGF? OR EGF? OR PDGF OR CNTF OR IGF? OR BDNF)
L15          0 S L9 AND (FGF? (L) EGF? (L) PDGF (L) CNTF (L) IGF? (L) BDNF)
L16         49 S L14 AND REVIEW
L17         28 DUP REM L16 (21 DUPLICATES REMOVED)
L18         28 SORT L17 PY
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L19         27 S E1
L20         21 DUP REM L19 (6 DUPLICATES REMOVED)
L21        440 S L10 AND L9
L22      11273 S L10 OR L9
L23        440 S L10 (L) L9
L24         21 S L20 AND NEUR?
L25         21 SORT L24 PY
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=> d an ti so au ab pi l25 9 10 12 13 14

L25 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1996:123431 CAPLUS
DN 124:227790
TI Differentiation of the immortalized adult **neuronal** progenitor cell line HC2S2 into **neurons** by regulatable suppression of the v-myc oncogene
SO Proceedings of the National Academy of Sciences of the United States of America (1996), 93(4), 1518-23
CODEN: PNASA6; ISSN: 0027-8424
AU Hoshimaru, Minoru; Ray, Jasodhara; **Sah, Dinah W. Y.**; Gage, Fred H.
AB A regulatable retroviral vector in which the v-myc oncogene is driven by a tetracycline-controlled transactivator and a human cytomegalovirus minimal promoter fused to a tet operator sequence was used for conditional immortalization of adult rat **neuronal** progenitor cells. A single clone, HC2S2, was isolated and characterized. Two days after the addn. of tetracycline, the HC2S2 cells stopped proliferating, began to extend **neurites**, and expressed the **neuronal** markers tau, NeuN, **neurofilament** 200 kDa, and glutamic acid decarboxylase in accordance with the reduced prodn. of the v-myc oncoprotein. Differentiated HC2S2 cells expressed large sodium and calcium currents and could fire regenerative action potentials. These results suggest that the suppression of the v-myc oncogene may be sufficient to make proliferating cells exit from cell cycles and induce terminal differentiation. The HC2S2 cells will be valuable for studying the differentiation process of **neurons**.

L25 ANSWER 10 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1997:371172 CAPLUS
DN 127:93276
TI Bipotent progenitor cell lines from the human CNS
SO Nature Biotechnology (1997), 15(6), 574-580
CODEN: NABIF9; ISSN: 1087-0156
AU **Sah, Dinah W. Y.**; Ray, Jasodhara; Gage, Fred H.
AB Human central nervous system (CNS) cell lines would substantially facilitate drug discovery and basic research by providing a readily renewable source of human **neurons**. We isolated clonal human CNS

cell lines that had been immortalized with a tetracycline (Tc)-responsive v-myc oncogene; addn. of Tc to the growth medium suppressed the oncoprotein rapidly and virtually completely, allowing differentiation to proceed. Two classes of bipotent precursor cells were immortalized: the first class had a default differentiation pathway of **neurons** only, and the second class had a default differentiation pathway of **neurons** and astrocytes. We found that after exposure to different external signals in vitro, the environment is capable of redirecting the fate of a particular cell, even in the case of the bipotent precursor cell whose default differentiation pathway was **neurons** only. These data suggest that extrinsic cues can prevail over intrinsic determinants in directing cell fate in the human CNS.

L25 ANSWER 12 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1998:176007 CAPLUS
DN 128:229000
TI Development of human CNS cell lines and use to study CNS cell development, death, and abnormalities
SO PCT Int. Appl., 76 pp.
CODEN: PIXXD2
IN **Sah, Dinah W. Y.**; Gage, Fred H.; Ray, Jasodhara
AB Conditionally-immortalized human CNS progenitor cell lines are provided. Such cell lines, which may be clonal, may be used to generate **neurons** and/or astrocytes. Such cell lines and/or differentiated cells may be used for the development of therapeutic agents to prevent and treat a variety of CNS-related diseases. The cell lines are produced by transfecting CNS progenitor cells with oncogenes and growing the cells on polyornithine/laminin, polylysine/laminin, or fibronectin-treated surfaces in culture medium supplemented with proliferation-enhancing factors. Suitable oncogenes include v-myc, N-myc, c-myc, p53, SV40 large T antigen, polyoma large T antigen, E1a adenovirus, and the human papillomavirus E7 protein gene. Such cell lines and/or differentiated cells may also be used in assays and for the general study of CNS cell development, death and abnormalities. Examples of abnormalities include Alzheimer's disease, stroke, traumatic head injuries, and amyotrophic lateral sclerosis.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9810058	A1	19980312	WO 1997-US15442	19970902
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9743315	A1	19980326	AU 1997-43315	19970902
AU 727113	B2	20001130		
EP 925357	A1	19990630	EP 1997-941398	19970902
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001500727	T2	20010123	JP 1998-512817	19970902

L25 ANSWER 13 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1999:672982 CAPLUS
DN 131:269274
TI PNS cell lines and methods of use therefor
SO PCT Int. Appl., 84 pp.
CODEN: PIXXD2
IN **Sah, Dinah W. Y.**; Raymon, Heather K.
AB Conditionally-immortalized PNS progenitor cell lines are provided. Such cell lines, which may be clonal, may be used to generate **neurons** . The cell lines and/or differentiated cells may be used for the development of therapeutic agents to prevent and treat a variety of PNS-related diseases. The cell lines and/or differentiated cells may also be used in assays and for the general study of PNS cell development, death and abnormalities.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9953028	A1	19991021	WO 1999-US8167	19990414
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				

RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2325603	AA	19991021	CA 1999-2325603	19990414
AU 9936429	A1	19991101	AU 1999-36429	19990414
EP 1071750	A1	20010131	EP 1999-918545	19990414
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9909624	A	20010911	BR 1999-9624	19990414
JP 2002511248	T2	20020416	JP 2000-543576	19990414

L25 ANSWER 14 OF 21 CAPLUS COPYRIGHT 2002 ACS
AN 1999:577009 CAPLUS
DN 131:195449
TI Conditionally-immortalized human spinal cord cell lines and uses thereof in the diagnosis, treatment, and prevention of spinal cord-related diseases and injuries
SO PCT Int. Appl., 37 pp.
CODEN: PIXXD2
IN Li, Ronghao; **Sah, Dinah W. Y.**
AB Conditionally-immortalized human spinal cord cell lines are provided. In one aspect, the present invention provides methods for producing a conditionally-immortalized human spinal cord **neural** precursor cell, comprising the steps of: (a) transfecting human spinal cord cells, plated on a first surface and in a first growth medium that permit proliferation, with DNA encoding a selectable marker and an externally regulative growth-promoting gene; and (b) selecting the transfected cells on a second surface and in a second growth medium that permit attachment and proliferation, and therefrom producing said immortalized cell. In certain embodiments, the growth-promoting gene may be an oncogene, such as v-myc, and expression of the growth-promoting gene may, but need not, be inhibited by tetracycline. Such cell lines, which may be clonal, may be used to generate **neurons**, including motor **neurons**.
The cell lines and/or differentiated cells may be used for the development of therapeutic agents to prevent and treat a variety of spinal cord-related diseases and injuries. The cell lines and/or differentiated cells may also be used in assays and for the general study of spinal cord cell development and differentiation.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9945103	A2	19990910	WO 1999-US4535	19990302
WO 9945103	A3	19991125		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6225122	B1	20010501	US 1998-59790	19980413
CA 2322747	AA	19990910	CA 1999-2322747	19990302
AU 9930655	A1	19990920	AU 1999-30655	19990302
EP 1060245	A2	20001220	EP 1999-912239	19990302
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9908443	A	20020115	BR 1999-8443	19990302

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(FILE 'HOME' ENTERED AT 16:14:55 ON 17 SEP 2002)

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L2          10 DUP REM L1 (13 DUPLICATES REMOVED)
L3          10 SORT L2 PY
L4       31741 S MESENCEPHALON
L5         208 S L4 AND (NEUR? (S) PROGENITOR)
L6          87 DUP REM L5 (121 DUPLICATES REMOVED)
L7           5 S L6 AND IMMORTAL?
L8           5 SORT L7 PY
L9       11273 S NEUR? (S) PROGENITOR
L10        440 S L9 AND IMMORTAL?
L11        141 DUP REM L10 (299 DUPLICATES REMOVED)
L12        141 FOCUS L11 1-
L13          5 S L11 AND HC2S2
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=> d an ti so au ab pi l13 5

L13 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2002 ACS
AN 1997:568304 CAPLUS
DN 127:201026
TI Regulatable retroviral vector containing v-myc oncogene for **immortalization** of adult **neuronal progenitor** cells
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
IN Gage, Fred H.; Ray, Jasodhara; Hoshimaru, Minoru
AB A novel regulatable retroviral vector in which the v-myc oncogene is driven by a tetracycline-controlled transactivator and a human cytomegalovirus minimal promoter fused to tet operator sequence useful for **immortalization** of adult **neuronal progenitor** cells is provided. Producer cell lines which produce high titers of the recombinant retrovirus are also provided. This general method is exemplified by the retroviral vector LINXv-myc. **HC2S2** cells from adult rat hippocampus were infected with the retroviral vectors. **HC2S2** cells, derived from an **immortalized** **neuronal progenitor** cell, were differentiated into **neurons** after suppression of the v-myc oncogene.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9730168	A1	19970821	WO 1997-US2013	19970211
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5770414	A	19980623	US 1996-602203	19960220
CA 2242381	AA	19970821	CA 1997-2242381	19970211
AU 9722642	A1	19970902	AU 1997-22642	19970211
AU 721727	B2	20000713		
EP 892851	A1	19990127	EP 1997-906896	19970211
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000504584	T2	20000418	JP 1997-529408	19970211

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L12 ANSWER 12 OF 141 MEDLINE
AN 96202311 MEDLINE
TI Differentiation of the **immortalized** adult **neuronal** **progenitor** cell line HC2S2 into **neurons** by regulatable suppression of the v-myc oncogene.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Feb 20) 93 (4) 1518-23.
Journal code: 7505876. ISSN: 0027-8424.
AU Hoshimaru M; Ray J; Sah D W; Gage F H
AB A regulatable retroviral vector in which the v-myc oncogene is driven by a tetracycline-controlled transactivator and a human cytomegalovirus minimal promoter fused to a tet operator sequence was used for conditional

immortalization of adult rat **neuronal progenitor** cells. A single clone, HC2S2, was isolated and characterized. Two days after the addition of tetracycline, the HC2S2 cells stopped proliferating, began to extend **neurites**, and expressed the **neuronal** markers tau, NeuN, **neurofilament** 200 kDa, and glutamic acid decarboxylase in accordance with the reduced production of the v-myc oncoprotein. Differentiated HC2S2 cells expressed large sodium and calcium currents and could fire regenerative action potentials. These results suggest that the suppression of the v-myc oncogene may be sufficient to make proliferating cells exit from cell cycles and induce terminal differentiation. The HC2S2 cells will be valuable for studying the differentiation process of **neurons**.

L12 ANSWER 1 OF 141 CAPLUS COPYRIGHT 2002 ACS
AN 1997:568304 CAPLUS
DN 127:201026
TI Regulatable retroviral vector containing v-myc oncogene for **immortalization** of adult **neuronal progenitor** cells
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
IN Gage, Fred H.; Ray, Jasodhara; Hoshimaru, Minoru
AB A novel regulatable retroviral vector in which the v-myc oncogene is driven by a tetracycline-controlled transactivator and a human cytomegalovirus minimal promoter fused to tet operator sequence useful for **immortalization** of adult **neuronal progenitor** cells is provided. Producer cell lines which produce high titers of the recombinant retrovirus are also provided. This general method is exemplified by the retroviral vector LINXv-myc. HC2S2 cells from adult rat hippocampus were infected with the retroviral vectors. HC2S2 cells, derived from an **immortalized neuronal progenitor** cell, were differentiated into **neurons** after suppression of the v-myc oncogene.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9730168	A1	19970821	WO 1997-US2013	19970211
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5770414	A	19980623	US 1996-602203	19960220
CA 2242381	AA	19970821	CA 1997-2242381	19970211
AU 9722642	A1	19970902	AU 1997-22642	19970211
AU 721727	B2	20000713		
EP 892851	A1	19990127	EP 1997-906896	19970211
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000504584	T2	20000418	JP 1997-529408	19970211

L12 ANSWER 2 OF 141 CAPLUS COPYRIGHT 2002 ACS
AN 1999:20170 CAPLUS
DN 130:245944
TI The use of **neural progenitor** cells for therapy in the CNS disorders
SO CNS Regeneration (1999), 183-201. Editor(s): Tuszynski, Mark H.; Kordower, Jeffrey H. Publisher: Academic, San Diego, Calif.
CODEN: 67CYA3
AU Ray, Jasodhara; Palmer, Theo D.; Shihabuddin, Lamya S.; Gage, Fred H.
AB A review with 84 refs. In recent years a significant no. of neurol. diseases have been defined at the mol. level. Somatic gene therapy using genetically modified non-neuronal cells expressing therapeutic factors have been successfully used in animal models of neurodegenerative diseases. Ability to grow central nervous system (CNS)-derived **neural progenitor** cells has proven to be extremely useful to study a diverse phenomenon including the fate choice, differentiation, and synaptic maturation of cells. **Immortal** or perpetual cultures of **neural progenitor** cells implanted into the rodent brain survive, migrate, and integrate in the host cytoarchitecture. These cells can be genetically modified to express therapeutic gene products. The ability of the implanted cells to integrate in the host brain and express transgene products in situ offer potential approaches for gene therapy in certain CNS diseases. The

utility of this approach has already been explored in animal models of neurodegenerative diseases. This chapter reviews the recent advances made in understanding the nature and potentiality of **neural progenitor** cells in vitro and in vivo as well as their possible use for cell replacement and gene therapy.

L12 ANSWER 5 OF 141 MEDLINE
AN 95212010 MEDLINE
TI A short term analysis of the behaviour of conditionally **immortalized neuronal progenitors** and primary **neuroepithelial** cells implanted into the fetal rat brain.
SO BRAIN RESEARCH. DEVELOPMENTAL BRAIN RESEARCH, (1994 Dec 16) 83 (2) 197-208.
Journal code: 8908639. ISSN: 0165-3806.
AU Cattaneo E; Magrassi L; Butti G; Santi L; Giavazzi A; Pezzotta S
AB Conditionally **immortalized** (temperature-sensitive) striatal-derived **neuronal progenitor** cell lines and primary **neuroepithelial** cells were transplanted into the CNS of gestational day 15-16 rat fetuses using an 'in utero' surgical procedure. Each fetus received 2.5-3 x 10(4) donor cells previously labelled in vitro by incubation with 5-bromo-2'-deoxyuridine (BrdU). At 5 days following transplantation, 69% of the fetuses were still alive. Engrafted cells were detected by BrdU immunohistochemistry, and the appearance of the engrafted cells and the time course of Nestin and PCNA expression were measured at 6, 24, 64 h and 5 days after transplantation. The evolution of Large T-Antigen immunoreactivity in engrafted temperature-sensitive (ts) cells was also evaluated at the above time intervals. The results indicate that the majority of the implanted cells were aggregated into clusters 24 h after transplantation. These clusters were not visible at 6 h, when most of the cells were isolated. The clusters were located in both the ventricles and parenchyma. These findings were common to both ts cells and striatal primary **neuroepithelial** cells. At 64 h and 5 days, isolated cells associated with the germinal layer and scattered throughout the parenchyma were also found. In the clusters, Nestin expression decreased proportionally with time following transplantation. Furthermore, Large T-Antigen immunoreactivity disappeared from ts cells between 6 and 24 h after transplantation. Finally, measurements of the temporal evolution of PCNA expression within the clusters indicate a progressive reduction in the mitotic activity of the transplanted cells. The results demonstrate that striatal primary **neuroepithelial** cells and conditionally **immortalized neuronal progenitors** can survive, migrate and/or compartimentalize into clusters whilst changing their antigenic properties and ability to proliferate.

L12 ANSWER 7 OF 141 MEDLINE
AN 1998063822 MEDLINE
TI In vitro pattern formation during **neurogenesis** in **neuroectodermal progenitor** cells **immortalized** by p53-deficiency.
SO INTERNATIONAL JOURNAL OF DEVELOPMENTAL NEUROSCIENCE, (1997 Oct) 15 (6) 795-804.
Journal code: 8401784. ISSN: 0736-5748.
AU Schlett K; Herberth B; Madarasz E
AB In vitro **neural** differentiation was induced in a p53-deficient **immortalized neuroectodermal progenitor** cell line, NE-4C, by treatment with retinoic acid [K. Schlett and E. Madarasz (1997) J. **Neurosci**. Res. 47, 405-416]. Rearrangement of nestin filaments was an early marker of **neuron**-formation. The increase in **neurofilament** protein content was accompanied by a decrease in the expression of nestin filaments in induced precursors. Cells with astroglial features appeared with a delay of 4-5 days compared to the appearence of **neurons**. Future **neurons** were sorted out from the substrate-attached population of apparently non-induced cells. The sorting out of future **neurons** resembled the separation of **neural** precursors in vivo. The continuous changes in the shape and also in the position of the cells resulted in the formation of characteristic morphological patterns. On the basis of morphological changes, five characteristic stages of in vitro **neural** differentiation were distinguished. The analysis of the morphological

changes revealed that cell-to-cell interactions played an essential role in the cell fate decision made by induced precursors. Our observations indicate that the NE-4C cell line can serve as an in vitro model to investigate some early steps of **neurogenesis**.

L12 ANSWER 10 OF 141 CAPLUS COPYRIGHT 2002 ACS
AN 1994:266389 CAPLUS
DN 120:266389
TI **Immortalization** of neural cells with the c-myc and N-myc proto-oncogenes
SO NeuroProtocols (1993), 3(3), 200-13
CODEN: NEPREV; ISSN: 1058-6741
AU Bernard, Ora
AB A review, with 59 refs. The c-myc and the N-myc proto-oncogenes were employed to **immortalize neural progenitor** cells. Infection of neural precursors isolated from the mouse at the 10th day of embryonic development (E10) with myc-contg. retroviruses resulted in **immortalized** cell lines representing bipotential E10 neuroepithelial cells. These cell lines have the capacity to differentiate into both glial and neuronal cells either spontaneously in the case of the Zen(myc) cell lines or after addn. of fibroblast growth factor to the Dol(myc) cell lines. Infection of migrating neural crest cells with the myc retroviruses gave rise to three different types of **immortalized** cell lines: (i) cell lines resembling freshly isolated neural crest cells; (ii) cell lines that can differentiate into cells expressing Schwann cell markers when grown at high cell concns.; and (iii) cell lines that have the ability to differentiate in culture to process-bearing cells which expressed neuronal markers or have the characteristics of Schwann cells. Olfactory epithelial cell lines were generated by infection with Zen retrovirus bearing the N-myc proto-oncogene. Some of the cell lines resemble basal cells and others grow as bipolar cells resembling neurons and expressing the neuronal marker neurofilaments.

L12 ANSWER 13 OF 141 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:519851 BIOSIS
TI Growth factor addition influences **immortalized neural progenitor** cell proliferation and differentiation.
SO Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 941. print. Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001
ISSN: 0190-5295.
AU Teppen, T. L. (1); Peterson, D. A. (1)
AB **Neural** stem cells provide a potential therapy to repair **neuronal** loss experienced with **neurodegenerative** disease. C17-2 **neural progenitor** cells (provided by E.Y. Snyder) are reported to display lower mitotic activity when cultured in completely defined media (N2) compared to serum supplementation. Addition of specific growth factors has been reported to enhance proliferation. To evaluate the effect of the growth factors bFGF and BDNF on proliferation and differentiation, C17-2 cells were cultured in defined (N2) media alone or supplemented with bFGF, BDNF or bFGF+BDNF in the absence of serum. Cell number and phenotype were compared with cells maintained in serum supplemented media. Cells cultured in defined medium supplemented with bFGF, BDNF or both factors together displayed a change in morphology compared with cells cultured in serum. While serum supplementation produced a cell monolayer, the addition of growth factors resulted in the formation of cell clusters appearing as proliferative cell colonies. A higher level of proliferation was observed across the bFGF and bFGF+BDNF conditions than with BDNF alone. Previous findings that bFGF and BDNF display a synergistic interaction were not observed. All defined media conditions appeared to have a lower growth rate (between 39-65%) than that of serum containing cultures. Results suggest that both bFGF and BDNF are mitogens for C17-2 cells. We are currently assessing the effect of growth factors on **neuronal** differentiation. These findings suggest C17-2 cells have the potential to proliferate and differentiate in response to specific environmental manipulations.

L12 ANSWER 14 OF 141 MEDLINE
AN 1998031251 MEDLINE

TI **Immortalized neural progenitor** cells for CNS gene transfer and repair.
SO TRENDS IN NEUROSCIENCES, (1997 Nov) 20 (11) 530-8. Ref: 58
Journal code: 7808616. ISSN: 0166-2236.
AU Martinez-Serrano A; Bjorklund A
AB **Immortalized** multipotent **neural** stem and **progenitor** cells have emerged as a highly convenient source of tissue for genetic manipulation and ex vivo gene transfer to the CNS. Recent studies show that these cells, which can be maintained and genetically transduced as cell lines in culture, can survive, integrate and differentiate into both **neurons** and glia after transplantation to the intact or damaged brain. **Progenitors** engineered to secrete trophic factors, or to produce **neurotransmitter**-related or metabolic enzymes can be made to repopulate diseased or injured brain areas, thus providing a new potential therapeutic tool for the blockade of **neurodegenerative** processes and reversal of behavioural deficits in animal models of **neurodegenerative** diseases. With further technical improvements, the use of **immortalized neural progenitors** may bring us closer to the challenging goal of targeted and effective CNS repair.

L12 ANSWER 16 OF 141 MEDLINE
AN 97084035 MEDLINE
TI Conditionally **immortalized neural progenitor** cell lines integrate and differentiate after grafting to the adult rat striatum. A combined autoradiographic and electron microscopic study.
SO BRAIN RESEARCH, (1996 Oct 21) 737 (1-2) 295-300.
Journal code: 0045503. ISSN: 0006-8993.
AU Lundberg C; Field P M; Ajayi Y O; Raisman G; Bjorklund A
AB **Neural progenitor** cell lines, generated by conditional **immortalization** from the embryonic CNS, have previously been shown to survive and integrate after transplantation to the adult brain. The present study was designed to investigate the in vivo differentiation and morphological features of grafted **neural progenitors** using combined autoradiography and transmission electron microscopy of two temperature-sensitive **neural progenitor** cell lines, HiB5 and ST14A, labeled with 3H-thymidine prior to grafting. Two weeks after transplantation to the striatum the cells were found dispersed over an area extending about 1.5 mm from the injection site. Labeled cells located within the myelinated fiber bundles of the internal capsule were closely associated with myelinated axons and presented profiles similar to oligodendrocytes, while most of the grafted cells in the grey matter had morphological features of astroglia. Some labeled cells occurred also in close association with small blood vessels, morphologically resembling host pericytes. The results show that the **immortalized neural progenitors** can differentiate into mature glial cells, including astrocytes, oligodendrocytes and pericytes, after implantation into the adult striatum. The ability of the cells to become fully integrated with the resident glial population suggests that they will be highly useful as vehicles for intracerebral transgene expression in ex vivo gene transfer.

L12 ANSWER 17 OF 141 MEDLINE
AN 94376081 MEDLINE
TI FGF and EGF are mitogens for **immortalized neural progenitors**.
SO JOURNAL OF NEUROBIOLOGY, (1994 Jul) 25 (7) 797-807.
Journal code: 0213640. ISSN: 0022-3034.
AU Kitchens D L; Snyder E Y; Gottlieb D I
AB Individual **neural progenitors**, derived from the external germinal layer of neonatal murine cerebellum, were previously **immortalized** by the retrovirus-mediated transduction of avian myc (v-myc). C17-2 is one of those clonal multipotent **progenitor** cell lines (Snyder et al., 1992, Cell 68: 33-51; Ryder et al., 1990, J. **Neurobiol**. 21:356-375). When transplanted into newborn mouse cerebellum (CB), the cells participate in normal CB development; they engraft in a cytoarchitecturally appropriate, nontumorigenic manner and differentiate into multiple CB cell types (**neuronal** and glial) similar to endogenous **progenitors** (Snyder et al., 1992, as

above). They also appear to engraft and participate in the development of multiple other structures along the **neural** axis and at multiple other stages (Snyder et al., 1993, Soc. **Neurosci**. Abstr. 19). Thus conclusions regarding these **immortalized progenitors** may be applicable to endogenous **neural progenitors** in vivo. To help identify and analyze factors that promote differentiation of endogenous **progenitors**, we first investigated the ability to maintain C17-2 cells in a defined, serum-free medium (N2). The cells survive in vitro in N2 but undergo mitosis at a very low rate. Addition of epidermal growth factor (EGF), however, either from mouse submaxillary gland or the human recombinant protein, appreciably stimulates thymidine incorporation and cell division approximately threefold. Basic fibroblast growth factor (bFGF) is an even more potent mitogen, promoting thymidine incorporation, cell division, and a net increase in cell number equal to that in serum. Both EGF and bFGF are active at very low nanomolar concentrations, suggesting that they interact with their respective receptors rather than a homologous receptor system. The findings demonstrate that C17-2 cells can be maintained and propagated in a fully defined medium, providing the basis for analysis of other growth and differentiation factors. That EGF and particularly bFGF are mitogenic for these cells is in accord with recent observations on primary **neural** tissue (Reynolds and Weiss, 1992, Science 255:1707-1710; Kilpatrick and Bartlett, 1993, **Neuron** 10:255-265; Ray et al., 1993, Proc. Natl. Acad. Sci. USA 90:3602-3606) suggesting that bFGF and EGF responsiveness may be fundamental properties of **neural progenitors**.

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